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ORIGINAL PAPER

Context-dependent acceptance of non-nestmates in a primitively eusocial insect

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Abstract The benefits of cooperation are essential in driving group formation. However, an individual can gain significant benefits by acting selfishly at a substantial cost to others in the group. Thus, group members must find a balance between accepting and rejecting potential new members. Here, I explore the factors that mediate acceptance of non-related individuals during the period of group establishment in the primitively eusocial wasp *Mischocyttarus mexicanus*. In this species, group composition changes during establishment, with non-related females (non-nestmates) sometimes accepted into a foreign colony. By experimentally introducing non-nestmates to newly established colonies, I test the hypothesis that acceptance threshold of nestmates towards non-nestmates shifts depending on the ecological context, as predicted by the Optimal Acceptance Threshold Model. I explored how non-nestmate age (young vs. old), stage of colony establishment (early vs. late), initial behavior of the non-nestmates (non-aggressive vs. aggressive), and the behavioral response by nestmates (non-aggressive vs. aggressive) affected the rates of acceptance. My results show an effect of both non-nestmate age and stage of colony development on non-nestmate acceptance. Young non-nestmates were more frequently accepted in early than in late colonies. Late colonies more frequently rejected both young and old non-nestmates, suggesting a cost of accepting potential usurpers into late colonies. Surprisingly, non-nestmate aggressive behavior did not have a direct effect on their acceptance, but it triggered an aggressive response from nestmates. These findings reveal a shift in the acceptance threshold, suggesting an effect of the social context and the specific needs of a colony on non-nestmate acceptance.

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Introduction

Group formation has evolved independently across the animal kingdom because of the fitness benefits of cooperative behavior to each member (Brown 1987; Dugatkin 1997; Solomon and French 1997; Koenig and Dickinson 2004; Foster 2009). However, group members can often gain direct benefits from selfish behavior at the expense of others, indicating a potential significant cost to group formation (West et al. 2007). Given this potential conflict, how do group members decide on whom to include in their group? This question has been studied extensively in highly eusocial insects, such as ants and honeybees, because they represent the most extreme example of cooperative behavior while having high conflict among group members (Heinze 2010). That is, in these highly eusocial insects, only one or a few females reproduce with the remaining females foregoing direct reproduction to raise non-descendent offspring (Wilson 1971). However, because highly eusocial groups have a defined queen since nest foundation, permanent caste systems, and a strict colony structure (Nonacs and Reeve 1995), they may not be ideal for understanding the factors that mediate the acceptance of unrelated non-nestmates into a group during the initial period of group establishment.

Alternatively, colonies of primitively eusocial insects without permanent castes, such as paper wasps, have an extended period of group establishment (approximately 8 weeks), which provides a unique opportunity to study the process of group formation in field conditions (Zanette and Fields 2011). During this initial period of establishment, individuals exhibit behavioral plasticity and groups frequently change in their social hierarchy as well as the number of members (Zanette and Field 2011; Mora-Kepfer 2011). These changes in group composition are influenced by environmental conditions and the social interactions among colony members (Reeve 1991; Starks 1998; Mora-Kepfer 2011). During nest construction and colony formation, many females are capable of reproducing and thus compete for the reproductively dominant position (Itô 1993; Gunnels 2007; Gunnels et al. 2008; Mora-Kepfer 2011). As a result, only one female becomes reproductively and socially dominant, while the remaining females act as subordinates (Reeve 1991). Because of this plasticity in reproduction, social behavior, and group composition during nest formation, newly established colonies of primitively eusocial wasp colonies are an ideal model system to study the factors that influence group formation.

During colony establishment, nest-switching is frequent with individual females trying to join other colonies and group members must decide to accept or reject approaching non-nestmates (Sumner et al. 2007; Mora-Kepfer 2011). This decision is especially critical in this establishment phase when all work is performed by the initial colony foundress/es before the emergence of adult offspring (Reeve 1991). It might favor the colony to accept non-nestmates as subordinate workers during this specific period. However, if non-nestmates are accepted, they may also attempt to take over the reproductively dominant role, commit brood theft, or cannibalize the colony's immature offspring (Klahn 1988; Clouse 1995). After colony establishment, the group's composition is maintained by recognizing nestmates from non-nestmates using durable chemical cues that consist of a cuticular hydrocarbon profile specific to each colony (Waldman 1987; Singer and Espelie 1992; Lorenzi et al. 1996; Sherman et al. 1997; Vásquez and Silverman 2010; Penn and Frommen 2010). Nestmates compare the learned templates from their colony as adults to the hydrocarbon cues on other wasps (Gadagkar 1985; Gamboa et al. 1986a, b; Mateo 2004). Each colony acquires its specific hydrocarbon profile gradually within the first days of establishment (Arathi et al. 1997; Panek et al. 2001). Similarly, after an adult wasp ecloses and ages, it acquires the specific profile of its colony and is recognized by its nestmates. However, recognition of non-nestmates may not always cause their rejection (Reeve 1989), and, in certain social contexts, it may be beneficial for nestmates to accept non-nestmates into their colony (Kûdo et al. 2007).

The Optimal Acceptance Threshold Model posits that acceptance thresholds should not be fixed but instead shift as a response to changes in ecological conditions (Reeve 1989). This model assumes higher similarity in the cues among nestmates when compared to non-nestmates, and that the acceptance threshold depends on the fitness consequences of accepting and rejecting nestmates and non-nestmates. Studies in paper wasps and honeybees provide support for this model as a given non-nestmate may be accepted in certain social contexts but rejected in others, suggesting that shifts in non-nestmate acceptance are adaptive (Reeve 1989; Gamboa et al. 1991a, b; Starks et al. 1998; Downs and Ratnieks 2000; Couvillon et al. 2009). However, these studies were carried out in mature colonies with well-established hierarchies and multiple generations of adult offspring. Considering the significance of decisions to accept or reject potential nestmates, surprisingly little is known about the factors that influence non-nestmate acceptance during the initial and critical period of colony establishment.

Here, I take advantage of the primitively eusocial paper wasp, *Mischocyttarus mexicanus*, to test the hypothesis that non-nestmate acceptance is context-dependent during colony formation and establishment in. Further, to determine the factors that mediate the acceptance of non-nestmates, I experimentally introduced unrelated individuals from different nests ("non-nestmates") to colonies during two distinct phases of nest establishment by foundresses: newly established colonies (early) and established colonies immediately before the emergence of adult offspring (late). I tested the effects of the age of non-nestmates, the stage of colony development, initial behavior of non-nestmates, and behavioral response of nestmates during the introduction. According to the Optimal Acceptance Threshold Model, the acceptance threshold for non-nestmates should exhibit plasticity and shift depending on the ecological context and needs of the establishing colony. Alternatively, the acceptance threshold could be fixed, remaining constant throughout colony establishment until the emergence of the first adult offspring.

Methods

Study system

The primitively eusocial wasp *M. mexicanus* is native to the Bahamas, Puerto Rico, Cuba, and the southeastern USA, with a recent expansion to Texas (Carpenter et al. 2009). The experimental colonies used in this study belong to a subtropical population in Kendall Indian Hammocks, a Miami-Dade County park in Kendall, South Florida (25°69′N, 80°38′W). This subtropical population has the advantage of lacking seasonal changes shown to influence group formation in this species (Gunnels 2007). Instead, colonies are active and asynchronous in different stages throughout the year. Females are highly variable in reproduction and behavior during the establishment period before the emergence of the first adult offspring (Mora-Kepfer 2011). After colony establishment, one female becomes the primary reproductive and the other females become subordinates (Röseler 1991). Nest switching is frequent and females shift between solitary and group founding strategies, and more than one female within a colony can be reproductively viable (Litte 1977; Hermann et al. 1985; Clouse 1995, 2001; Gunnels 2007; Gunnels et al. 2008).

Non-nestmate introduction trials

For the experimental trials, I exclusively selected recently established colonies that had yet to produce adult offspring. The average duration of the adult offspring pre-emergence phase is 55 days in *M. mexicanus* (Litte 1977). Therefore, I chose two types of experimental colonies: "early colonies" and "late colonies." Early colonies ($N=42$) were within the first week of being established and consisted of a foundress or small group of foundresses, eggs, and first instar larvae. Late colonies ($N=44$) were approximately 5 weeks old and contained eggs, larvae, and pupae soon to emerge as adults. Established colonies with adult offspring were excluded.

I selected adults from marked nests as non-nestmates and introduced them into the experimental early and late colonies. To insure that non-nestmates were unrelated and did not have a similar hydrocarbon profile to the experimental colonies, I collected these non-nestmates in Matheson Hammock Park, which is 15 km away from Kendall Indian Hammocks. These two parks are surrounded by urban areas making it difficult for individuals to find nesting sites outside of their respective populations. Further, I collected two types of non-nestmates: "young non-nestmates" and "old non-nestmates". Young non-nestmates were collected the day they emerged as adults and introduced within 48 h, to insure they had not yet acquired the specific hydrocarbon profile of their colony (Panek et al. 2001; Lorenzi et al. 2004). I selected the old non-nestmates by marking newly emerged females, and collected and introduced them 10 days later to insure they had acquired their colony's hydrocarbon profile.

A trial consisted of one behavioral interaction between an experimental colony's nestmates and the introduced non-nestmate. The introduction protocol was as follows: I first isolated each non-nestmate in a cooler. When the wasp became inactive, I used the tip of an insect pin to paint its thorax with Testors® enamel paint for individual identification. When the wasp became active again, I introduced a thin wooden stick to the container and the wasp grasped the stick. I then placed the non-nestmate in contact with an experimental colony, allowing the non-nestmate, without prodding, to walk onto the experimental nest. This method avoided manipulation of wasps with forceps that might affect their behavior. I excluded events when the non-nestmate wasp did not voluntarily walk in proximity of the experimental nest.

Aggressive interaction scoring

I videotaped each interaction initiated by a non-nestmate and the response of the experimental colony nestmates ($N=86$). Interactions initiated by colony nestmates were excluded because they only occurred on four occasions. From the recordings, I timed the duration and categorized the outcome of each interaction as acceptance if the non-nestmate was allowed to remain in the experimental nest or rejection if the non-nestmate was physically forced off the nest. Additionally, I quantified the number of nestmates interacting with the non-nestmate. To determine the number of females of each experimental nest, I performed night censuses when all colony nestmates were present in their nest.

For each interaction, I categorized the initial behavior of the introduced non-nestmate (non-aggressive vs. aggressive) and the behavioral response of the experimental colony nestmates (non-aggressive vs. aggressive response) by using established ethograms (Itô 1993; O'Donnell 1998). For instance, if the interacting wasps antennated or did not have any physical contact, the interaction was classified as non-aggressive behavior. In contrast, if the interaction consisted of physical attacks such as biting, grappling, or physically forcing the non-nestmate off the nest, the behavior was classified as aggressive.

I assigned the aggression level of the non-nestmate and the colony nestmate for each interaction based on the aggression score of 0 to 4 established by Stuart and Herbers (2000). A score of 0 was assigned if interactions included only grooming and/or antennation. A score of 1 was assigned when wasps exhibited mild aggression such as pushing and occasional biting. A score of 2 was given if a wasp exhibited aggression such as biting and chasing. A score of 3 was assigned in interactions where there was physical dragging and a wasp was forced off the nest. Finally, a score of 4 was assigned to interactions in which a wasp tried to kill a non-nestmate by biting, stinging, and grappling. The assignment of aggression scores was done blind to the introduction treatment of each videotaped interaction.

Testing the effect of non-nestmate age and colony stage

I examined the effects of the age of non-nestmates (young vs. old) and stage of the experimental colony (early vs. late) on the acceptance of non-nestmates. My experiment consisted of four treatments: (1) introduction of a young non-nestmate into an early colony, (2) introduction of a young non-nestmate into a late colony, (3) introduction of an old non-nestmate into an early colony, and (4) introduction of an old non-nestmate into a late colony. To ensure that each interaction outcome was an independent event, I used each non-nestmate and each experimental colony only once, for a total of 86 interactions/colonies. As a control, I removed one resident wasp from its natal colony using the same methodology described above. I then re-introduced the resident back into its own colony. I repeated this control treatment for 10 young residents and 11 old residents during the same months I performed the experimental introductions and at 21 unique nests.

Statistical analyses

Because of the discrete nature of this data set, as well as not meeting the assumptions of normality and equality of variances, I used a specific type of logistic regression: Generalized Linear Models (GLM) with a binomial error structure (Nelder and Wedderburn 1972; McCulloch 2000). I specifically tested for the effects of non-nestmate age (young vs. old), stage of colony development (early vs. late), and non-nestmate aggression (aggressive vs. non-aggressive) in the acceptance of a non-nestmate.

For all GLM, I tested for the main effect of each of these discrete variables and the interaction among them on the binary response of either acceptance or rejection of non-nestmates. I then used the Akaike Information Criteria (AIC) to determine the most parsimonious model in the candidate set (Akaike 1973). The AIC value for each model, i, was calculated and was rescaled to ΔAIC ($\mathrm{AIC}_i - \mathrm{AIC}_{\mathrm{min}}$) to choose the best fit model and avoid the effect of sample size and arbitrary constraints (Caswell 2001; Burnham and Anderson 2004). In a candidate set, the models with $\Delta\mathrm{AIC} \leq 2$ are well supported and the simplest model is the best fitted. Full details on selecting the best-fit model are described in Burnham and Anderson (2004). GLM analyses were performed using the software R (R Development Core Team 2009).

I tested the effect of both non-nestmate age and colony stage on differences in the acceptance threshold by comparing the proportion of accepted non-nestmates among the four treatment groups using replicated G-tests of goodness-of-fit (Sokal and Rohlf 1995). Subsequently, I performed pairwise comparisons to determine differences between treatments. To test for the effect of the total number of colony nestmates and the number of nestmates present during introductions on non-nestmate acceptance, I performed Nominal Logistic Regressions.

Using Mann–Whitney U tests, I determined differences in aggression levels: towards old and young non-nestmates and between nestmates in early and late colonies. Kruskal–Wallis tests were performed to determine differences in aggression levels among (1) nestmates and (2) non-nestmates in the four introduction treatments with subsequent pairwise comparisons. SPSS 17 was used to analyze behavioral interactions (SPSS, Inc. 2008).

Results

Non-nestmate acceptance

From the nine candidate GLM that explained non-nestmate acceptance, model 8 was the best fit, supported by a value of $\Delta\mathrm{AIC}=0$ (Table 1). This model detected significant main effects of non-nestmate age and colony stage on non-nestmate acceptance, but did not find a significant interaction between non-nestmate age and colony stage (Table 2). Surprisingly, non-nestmate aggression did not have an effect on non-nestmate acceptance. The other models with a $\Delta\mathrm{AIC}<0.5$ (model 9, model 4, and model 1) show similar results as model 8.

Table 1 Comparison of models explaining non-nestmate acceptance in a foreign colony by non-nestmate age (A), non-nestmate aggression (N), and colony stage (S) (generalized linear models with a binomial error structure). The main effect between variables (+), the interaction between variables (x), and both main effect and interactions between variables were tested. The Akaike Information Criteria (AIC) was calculated for each model i. The most parsimonious model was selected by using the lowest ΔAIC ($\mathrm{AIC}_i - \mathrm{AIC}_{\mathrm{min}}$). Models 2–9 were obtained by simplifying model 1 step by step, so variables were subtracted in each consecutive model. Model 8 is the best fit model with a ΔAIC of 0

Model i	Variables	AIC	ΔAIC
M1	A+N+S+AxN+NxS+AxS+AxNxS	86.19	0.3
M2	A+N+S+AxN+NxS+AxS	87.96	2.07
M3	A+N+S+AxN+NxS	88.6	2.71
M4	A+N+S+AxN+AxS	85.99	0.1
M5	A+N+S+NxS+AxS	87.73	1.84
M6	A+N+S+AxN	86.7	0.81
M7	A+N+S+NxS	87.41	1.52
M8	A+N+S+AxS	85.88	0
M9	A+N+S	85.92	0.03

Non-nestmate acceptance differed significantly among the four introduction treatments ($G=82.26$, $df=4$, $P<0.001$, $N=84$, Fig. 1). Subsequent pairwise comparisons revealed significant differences in non-nestmate acceptance between young non-nestmates accepted into early nests in comparison to the other three treatments. The majority of young non-nestmates introduced to early colonies were accepted (62 %). The other three treatments resulted in significantly lower acceptance rates: 9 % of old non-nestmates were accepted by late colonies, 18 % of young nestmates were accepted by late colonies, and 5 % of old non-nestmates were accepted by early colonies (Fig. 1, $N=84$). In the control procedure, where nestmates were reintroduced to their own colony, all nestmates were

Table 2 Effect of non-nestmate age, colony stage, non-nestmate aggression, and interactions between non-nestmate age and colony stage on non-nestmate acceptance for the best fit model (model 8, see Table 1). $N=84$ interactions between a non-nestmate and colony nestmates. Italic emphasis indicates a significant effect of the tested variable

Variables	DF	Model 8 Residuals	DF residuals	*P*
Non-nestmate age	1	10.69	81	**0.001**
Colony stage	1	5.15	80	**0.02**
Non-nestmate aggression	1	0.69	82	0.4
Interaction age and stage	1	2.03	79	0.15

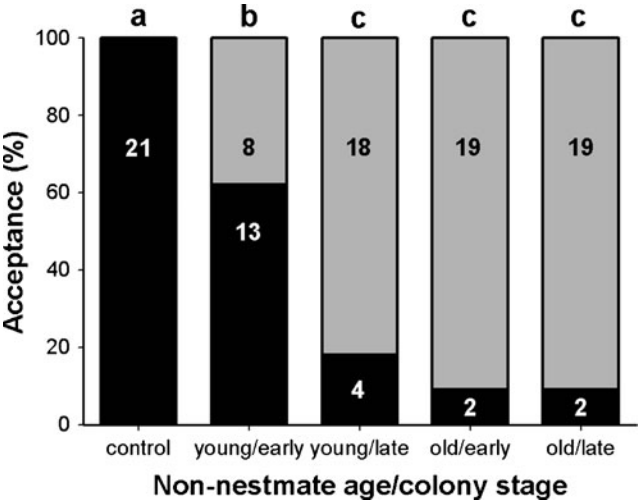

Fig. 1 Percentage of acceptance (*black bars*) and rejection (*gray bars*) for control and the four introduction treatments: (1) young non-nestmates introduced into early colonies, (2) young non-nestmates introduced into late colonies, (3) old non-nestmates introduced into early colonies, and (4) old non-nestmates introduced into late colonies. The control treatment consisted of re-introducing a wasp into its own nest after being marked. *Bars topped by different letters* differ significantly at $P<0.005$, Replicated G tests of goodness of-fit

accepted back into their own nest by their colony nestmates (Fig. 1, $N=21$).

The number of nestmates present at the time of the introduction did not affect non-nestmate acceptance ($\chi^2=0.812$, $P=0.84$, $R^2=0.01$, $N_{\text{colonies}}=71$). Likewise, total colony size (including nestmates not present at the nest at the time of the introduction) did not affect on non-nestmate acceptance ($\chi^2=3.51$, $P=0.78$, $R^2=0.003$, $N_{\text{colonies}}=81$).

Aggressive interactions

Independent effects of non-nestmate age and colony stage

Old non-nestmates approaching nestmates of a foreign colony showed higher levels of aggression than young non-nestmates (Mann–Whitney $U=1{,}067.5$, $df=1$, $P=0.04$, $N=84$). As a response, nestmates responded with higher levels of aggression towards old non-nestmates than young non-nestmates (Mann–Whitney $U=1{,}144.5$, $df=1$, $P=0.028$, $N=85$).

Non-nestmates exhibited only mild aggression toward colony nestmates in both early and late colonies (Mann–Whitney $U=748$, $df=1$, $P=0.14$, $N=84$). However, nestmates in late colonies responded to non-nestmates more aggressively than those in early colonies (Mann–Whitney $U=533$, $df=1$, $P=0.01$, $N=85$).

Joint effect of non-nestmate age and colony stage

The joint effects of non-nestmate age and colony stage on aggression levels differed among treatments. Young non-nestmates exhibited no aggression towards nestmates when introduced to early colonies, and mild aggression in the other three introduction treatments (Kruskal–Wallis test, $\chi^2=7.35$, $df=3$, $P=0.061$, $N=86$, Fig. 2a). Nestmates of early colonies responded by being mildly aggressive towards young non-nestmates but significantly more aggressive in the three other treatments (Kruskal–Wallis test, $\chi^2=18.70$, $df=3$, $P<0.001$, $N=85$, Fig. 2b).

Discussion

This study provides novel evidence for context-dependent acceptance of non-nestmates during the initial and critical period of nest construction and establishment of colonies in

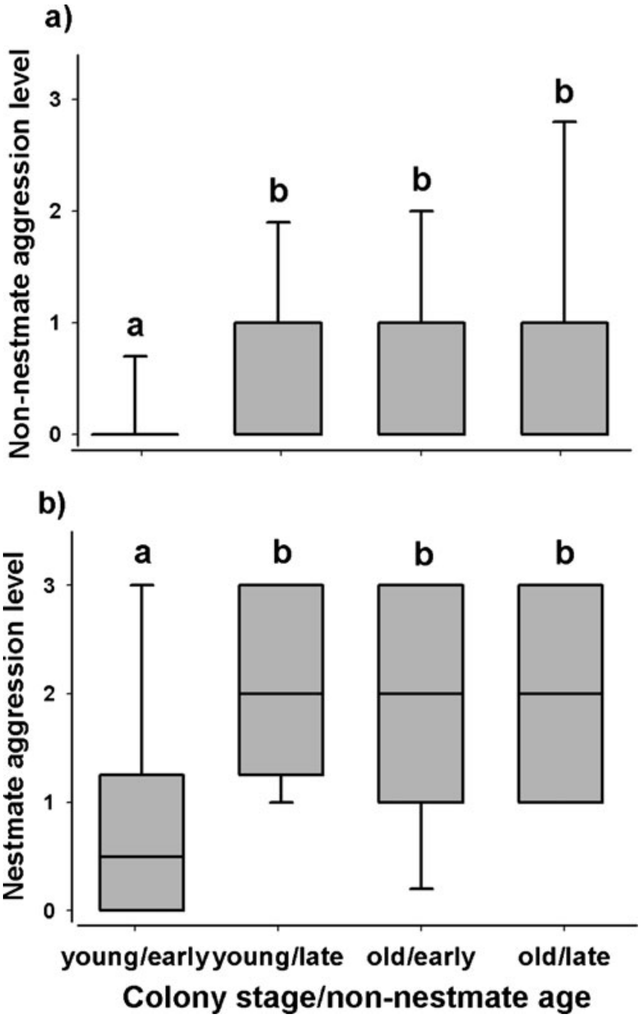

Fig. 2 Aggression levels between the introduced non-nestmate and colony nestmates in the four experimental treatments: **a** aggressive response of colony nestmates toward the introduced non-nestmate and **b** aggressive response of the non-nestmate toward colony nestmates. Box and whisker plots of aggressive categories show the median, 25th and 75th percentile (*box*) and 5th and 95th percentile (*whiskers*). *Bars topped by different letters* differ significantly at $P<0.001$, Kruskal–Wallis tests with subsequent pairwise comparisons

a primitively eusocial wasp. Non-nestmates were more frequently accepted in early versus late colonies. In addition, young non-nestmates were accepted more often than old non-nestmates, consistent with studies in well-established colonies with several generations of adults (Arathi et al. 1997; Panek et al. 2001). Therefore, my results indicate that the acceptance threshold shifted according to both non-nestmate age and colony stage. These results support the Optimal Acceptance Threshold Model, which predicts that selection favors shifts in the acceptance threshold within the same colonies under different ecological and/or social conditions (Reeve 1989). There might be a higher payoff to accepting non-nestmates that act as subordinate workers early during nest establishment when additional support for brood defense and nest construction is critical (Starks et al. 1998). In contrast, the benefits of recruiting additional workers after the colony has been well established may be diminished. My results support the idea that it may be advantageous for unrelated females to establish groups under certain social and ecological contexts to enhance the success of individuals in the group.

Non-nestmate age was a determining factor in their acceptance into foreign colonies. Acceptance of young non-nestmates may be more frequent because young *Mischocyttarus* females show limited ovary development until their second week of adult life (Molina and O'Donnell 2008; Mora-Kepfer 2011). In addition, young non-nestmates are inexperienced and typically non-aggressive (Arathi et al. 1997; Mora-Kepfer 2011). These characteristics, in turn, make them more likely to become subordinate workers rather than potential usurpers (Reeve 1991). In addition, accepting young non-nestmates may be beneficial because nests initiated by groups of foundresses are better defended against predators, parasitoids, and usurpation attempts than nests initiated by a single foundress (Litte 1977; Clouse 1995; Klahn 1988). Alternatively, high acceptance rate of young non-nestmates in early colonies may not be adaptative, but rather due to the inability of colony nestmates to recognize young non-nestmates that are in the process of acquiring a colony's chemical profile (Lorenzi et al. 1996; Panek et al. 2001; Lorenzi et al. 2004).

The acceptance of non-nestmates was also influenced by stage of colony development. Before the emergence of the first adult offspring that eventually become workers, the establishment of the colony depends exclusively on the foundress or foundresses (West 1967; Clouse 2001). Hence, the acceptance of non-nestmates as subordinate workers in early colonies may be advantageous to the colony by enhancing nest construction, foraging, and defense. In addition, the costs of accepting non-nestmates may also change with colony development. Foundresses in early colonies of *M. mexicanus* abandon early nests when threatened by usurpers instead of incurring a higher cost of defending the brood composed of eggs and very young larvae (Litte 1977). In contrast, significant investment in the brood close to eclosing as the first generation of adult workers may result in higher aggression and rejection of non-nestmates in late and well-established colonies. These results are consistent with another study that showed that aggressive behavior towards non-nestmates is more frequent in late colonies than early colonies (Judd 1998), suggesting that the risk of potential usurpation and brood theft may influence higher frequency of rejection of non-nestmates (Clouse 1995; Cervo and Lorenzi 1996; Klahn 1988). Finally, during early stages of colony establishment, nestmates are still acquiring and learning the chemical profile of their own colony (Singer and Espelie 1992, reviewed in Gamboa 2004), making it difficult to differentiate between nestmates and non-nestmates during this learning period. Late colonies, in contrast, have established colony profile so that group members can more readily recognize and reject non-nestmates (Gamboa et al. 1991b; Gamboa 1996).

It has been argued extensively that subordinate females sacrifice their own reproduction to help rear the offspring of the dominant individual in the group because of indirect fitness benefits of helping kin reproduce (Hamilton 1964; Trivers and Hare 1976; reviewed in Crozier and Pamilo 1996). Nonetheless, indirect fitness has failed to fully explain group living (Queller et al. 2000; Zanette and Field 2008; Leadbeater et al. 2010). In my study, the non-nestmates were selected from a park surrounded by urban areas and distantly located to insure that they were not related to and had different colony chemical profiles than members of the experimental colonies. A total of 24 % of the introduction experiments resulted in acceptance of unrelated non-nestmates, with the majority being young non-nestmates accepted into early colonies. Similarly, recent studies have unexpectedly found a high percentage of unrelated individuals in a colony and low levels of genetic relatedness among colony members in social insects. For instance, in several bee and wasp species, 25–35 % of the colony members are not related to their nestmates (Leadbeater et al. 2010, 2011; Queller et al. 2000; Soro et al. 2009; Zanette and Field 2008) and nest switching is frequent (reviewed in Reeve 1991; Sumner et al. 2007; Mora-Kepfer 2011). Thus, my ongoing work aims to determine the degree of relatedness within *M. mexicanus* colonies, as low relatedness among colony members might influence more frequent acceptance of non-nestmates.

Finally, context-dependent acceptance of non-nestmates may be influenced by behavioral interactions during an encounter between colony members and the approaching non-nestmate. Other studies have shown that non-nestmate acceptance is influenced by colony size and aggressive interactions between an approaching non-nestmate and the colony nestmates (Clouse 2001; Buczkowski and Silverman 2005). Although non-nestmate aggressive behavior did not have a direct effect on non-nestmate acceptance, aggressiveness in non-nestmates did trigger an aggressive response from resident nestmates. Further, an individual's prior experience may also influence aggressive behavior (Hsu et al. 2006). For example, in the Argentine ant, *Linepithema humile*, aggressive behavior towards non-nestmates increases with age and experience of the colony nestmates (Van Wilgenburg et al. 2010). Previous interactions of colony nestmates with potential usurpers may lead to elevated aggression in later encounters with non-nestmates. As a result, further encounters with aggressive non-nestmates may be perceived as a usurpation threat to the colony.

Conclusions

Overall, my results suggest plasticity in the response of colony members towards potential joiners. However, it remains unclear why a female would rather join a colony of non-relatives to become a subordinate worker instead of remaining in her natal nest or founding a new nest. Unrelated females may join foreign colonies because of the potential for future inheritance of the nest (Queller et al. 2000; Leadbeater et al. 2011) or opportunities to enhance direct fitness by becoming reproductive (Cant and Field 2001; Field et al. 2006; Leadbeater et al. 2010).

My findings also raise interesting questions about the mechanisms that underlie non-nestmate acceptance. Plasticity in the acceptance threshold may depend on the trade-off between the fitness costs of recognition errors such as accepting non-nestmates versus those of rejecting own nestmates (Gamboa et al. 1991b; Panek et al. 2001; Starks et al. 1998). An unexplored question of recognition systems is the potential effect of changes in the chemical profile throughout colony development on the acceptance of non-nestmates. The chemical profile is known to change within colonies over time (Vander Meer et al. 1989), so recognition cues may also change throughout the different stages of the colony cycle. In addition, nestmate recognition mechanisms may vary depending on colony size, as highly eusocial insect societies are composed of thousands of individuals, compared to hundreds or fewer individuals in primitively eusocial societies (Wilson 1971). For instance, a recent model in honeybees, a highly eusocial species, proposed that effective nestmate recognition can be achieved collectively even if individual members of the colony are poor recognizers (Johnson et al. 2011). However, strong evidence demonstrates that discrimination by guards at the entrance of honey bee and stingless bee colonies is critical when approached by a conspecific non-nestmate. (Downs and Ratnieks 2000; Couvillon et al. 2012, 2013). Thus, context has a significant effect in the ability to make appropriate recognition decisions (Couvillon et al. 2013). In primitively eusocial colonies, early groups are composed only of a few individuals and so strong nestmate recognition is essential in maintaining group stability when approached by potential joiners or usurpers. Future studies comparing recognition systems across different levels of sociality in insects would provide key insights into the mechanisms that influence context-dependent acceptance of unrelated individuals in animal groups.

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